ENVIRONMENTAL PROTECTION AGENCY

40 CFR Parts 798 and 799

[OPTS-46017A; FRL 3660-1]

RIN 2070-AB94

Mouse Visible Specific Locus Test Requirement; Final Amendment In Test Rules

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: EPA is issuing a final rule amending the requirement for the mouse visible specific locus test (MVSL) allowing sponsors of tests conducted under section 4 of the Toxic Substances Control Act (TSCA), to choose either the MVSL or the mouse biochemical specific locus test (MBSL) intesting for heritable gene mutations in mammals when notified by EPA that such testing is necessary. EPA believes that both tests are comparable and acceptable for detecting heritable gene mutations in mammals. This action also promulgates the test guideline for the MBSL, specifying a reporting requirement of 51 months for the completion of testing for either the MVSL or MBSL, and specifying certain specimen retention requirements for the MBSL and MVSL.

DATES: This rule shall be effective on May 21, 1990. In accordance with 40 CFR 23.5, this rule shall be promulgated for purposes of judicial review at 1 p.m. eastern standard time on April 19, 1990.

FOR FURTHER INFORMATION CONTACT: Michael M. Stahl, Director, Environmental Assistance Division (TS-799), Office of Toxic Substances, Rm. E-543B, 401 M St. SW., Washington, DC 20460, (202) 554-1404, TDD: (202) 554-0551.

SUPPLEMENTARY INFORMATION: In the **Federal Register** of December 23, 1988 (53 FR 51847), EPA issued a proposed rule under TSCA section 4(a) to amend the requirement for the MVSL in the proposed test rule for triethylene glycol monomethyl, monoethyl, and monobutyl ethers, and in final test rules for: (1) Four fluoroalkenes, (2) oleylamine (Phase I and Phase II), (3) commercial hexane, (4) unsubstituted phenylenediamines, and (5) isopropanol to allow the test sponsors for these or future test rules to use either the MVSL or the MBSL in the testing of chemical substances when notified by EPA. This notice promulgates these amendments and additions, and responds to public comment on the proposal.

I. Introduction

In the **Federal Register** of May 23, 1985 (50 FR 21398), EPA issued a final rule requiring testing of diethylenetriamine (DETA), including the MVSL. This would be triggered by a positive result in the *Drosophila* sex-linked recessive lethal test. In the **Federal Register** of April 10,1986 (51 FR 12344), EPA proposed test standards and reporting requirements for the testing of DETA which were promulgated in the **Federal Register** of February 3, 1987 (52 FR 3230). The Synthetic Organic Chemical Manufacturers Association and Texaco Chemical Corporation challenged this final rule in the U.S. Court of Appeals for the Third Circuit (No. 87-3265), arguing that the MVSL, required by the rule, would be impossible to perform due to the unavailability of a laboratory to perform the test.

The MVSL requirement also appears in five other final test rules: Fluoroalkenes (June 8, 1987, 52 FR 21516); oleylamine Phase I (August 24, 1987, 52 FR 31962); commercial hexane (February 5, 1988, 53 FR 3382); unsubstituted phenylenediamines (November 30, 1989, 54 FR 49285); and isopropanol (October 23, 1989, 54 FR 43252). Persons subject to these rules did not challenge the MVSL requirement.

Since that time, EPA confirmed the report that DETA did not produce positive results as defined in 40 CFR 798.5275 in the sex-linked recessive lethal test in *Drosophila*, the triggering test for the MVSL. Therefore, the petitioners and EPA asked the Court to dismiss the DETA case. The Court subsequently dismissed the case. However, because EPA believed that the MVSL issue still needed to be addressed, EPA issued the MVSL proposed rule on December 23,1988, (53 FR 51847).

The MVSL rule proposed exemptions to certain Good Laborotory Practice Standards (GLPs). Specifically, EPA proposed that testing facilities conducting either the MVSL or MBSL be exempt from the provisions of §§ 792.190(a) and 792.195(b) and (c) of the GLP Standards. These exemptions were limited to the storage and retention of certain biological preparations. Since the time of the proposed MVSL rule, a rule amending the GLP Standards has been promulgated (August 17, 1989; 54 FR 34034). In this rule amending the GLP Standards, the requirement to retain biological specimens for 10 years has been modified. The GLP Standards now state that biological specimens need to be retained only until after quality assurance verification. Therefore, the

exemptions to the GLP Standards proposed in the MVSL rule are no longer necessary and are not being promulgated. However, EPA is promulgating the additional requirements to the GLP Standards proposed in the MVSL rule specific to the MVSL and MBSL tests, which require the testing laboratory to take and retain for 10 years 35–mm photographs (and negatives) of all mutant animals, their siblings, and their parents (for the MVSL), and of the starch-gels and electrofocussing columns exhibiting the migrating patterns obtained from all mutant animals, their siblings, and their parents (for the MBSL).

II. Response to Public Comments

In the MVSL proposed rule, EPA solicited public comment specific to the MVSL and MBSL assays.

Comments on the MVSL proposed rule were received from the American Industrial Health Council (AIHC), the Chemical Manufacturers Association (CMA), E.I. DuPont de Nemours & Company (DuPont), Monsanto Company (Monsanto), the American Petroleum Institute (API), and the Synthetic Organic Chemical Manufacturers Association (SOCMA). EPA's full response to public comments is available in the record established for this rulemaking and consists of two memoranda from Michael C.Cimino to Ray Locke:

(1) "Response to Comments on Proposed Rule Amending MVSL Requirement", May 25, 1989.

(2) "Additional Response to Comments on Proposed Rule Amending MVSL Requirement", August 18, 1989.

The following is a summary of major comments and EPA's responses.

1. *Laboratory availability.* A comment was made that there is insufficient laboratory availability for running either the MVSL or MBSL assay commercially.

EPA acknowledges that the commercial availability of the MBSL assay is limited. However, Research Triangle Institute (RTI) currently conducts the MBSL and expects to conduct the MVSL in the future, and both studies can be done using TSCA GLP Standards at RTI.

2. *Spontaneous mutation rate.* A comment was made that there is substantial uncertainty over the historical spontaneous mutation rate; and that the assumption that detectable mutation rates at different loci are equivalent is unverified.

EPA agrees that the low spontaneous mutation rates may pose a problem. More flexibility may be necessary here, even to the extent of omitting the requirement for quantifying the historical spontaneous frequency contained within the guideline. This would permit individual laboratories conducting the test to establish their own historical data bases, without the burden of attempting to achieve a target frequency. EPA will examine such requests, and their justification, on a case-by-case basis.

3. *Cost.* A comment was made that the high cost of the MBSL, $350,000, is unwarranted.

EPA has acknowledged in the proposed rule the financial burden that running the assay would present to industry. Nevertheless, EPA believes that the importance of this health effect endpoint, coupled with the fact that two or more positive gene mutation assays in the two lower tiers of the testing sequence would have been necessary to arrive at the trigger for specific locus testing, warrant the expense of generating the data. EPA will continue to examine the value of this test and the financial burden on a case-by-case basis.

4. *Test relevance.* A comment was made that neither the MVSL or MBSL test will produce information of relevance to quantitative risk assessment. Current data on the MBSL do not demonstrate that the assay generates data suitable for quantitative risk assessment. EPA should wait 5 years until the MBSL is better validated, or until new assay system(s) are developed to assess heritable gene mutagenicity.

EPA provided justification for the use of the MVSL for risk assessment in prior test rules (see the final rule for diethylenetriamine (February 3, 1987, 52 FR 3230)). The MBSL is expected to have at least equivalent relevance to that of the MVSL. Many MBSL loci currently screened in mice are homologous to human loci and therefore are useful for risk assessment. While EPA encourages continued investigation in developing alternative assay systems to assess potential heritable gene mutagenicity, presently there are no known or available strong alternatives to the MVSL and MBSL, and it is unlikely that a break through discovery and validation of another test will occur in the next 5 years.

5. *Test equivalence.* A comment was made that there is uncertainty over the equivalence of the MBSL and MVSL assays, due to the nonspecific morphological or behavioral variants possible in the MBSL.

EPA believes that such additional possibilities do not reduce the efficacy of the MBSL. It still retains its ability to detect chemically-induced heritable gene mutations in an *in vivo* mammalian system, and thus serves the same basic purpose as the MVSL.

6. *Test statistics.* A comment was made that there is uncertainty concerning the required population sizes and specific statistical methods to be employed for the MBSL assay, the power of the test, and the reliability of the design.

Many guidelines do not have specific statistical methods specified, since this is an area under development for many mutagenicity assays at the present time. EPA Therefore believes it is inappropriate to recommend specific statistical methods in this guideline. Appropriate methods should be selected by the laboratory.

7. *Test inflexibility.* A comment was made that EPA should allow increased flexibility in test procedures (e.g., starch-gel electrophoresis) and reporting deadlines. EPA should permit detection of mutant bands by all other "proven, comparable, biochemical methods", not just starch-gel electrophoresis. Also, EPA should allow extended deadlines for new laboratories.

EPA's position is that guidelines in general are designed to provide guidance relative to the current state of the art in the assay system. If a test sponsor desires to modify the study design, such flexibility is provided by commenting on specific proposed test rules or by using the procedure under 40 CFR 790.68.

III. Rulemaking Record

EPA has established a record for this rulemaking within each of the existing records for the remaining proposed and final TSCA section 4(a) test rules currently containing a requirement for the MVSL. They are: (1) Fluoroalkenes, OPTS–42002K; (2) Unsubstituted phenylenediamines, OPTS–42008G; (3) Oleylamine, OPTS–42061F; (4) Triethylene glycolmonomethyl, monoethyl, and monobutyl ethers, OPTS–42080F; (5) Commercial hexane, OPTS–42084J; and (6) Isopropanol, OPTS–42097C.

This record contains the information EPA considered in developing this final rule, and includes the following:

Supporting Documentation

(1) **Federal Register** notices pertaining to this rule, consisting of:

(a) Final test rules for diethylenetriamine (February 3, 1987, 52 FR 3230); fluoroalkenes (June 8, 1987, 52 FR 21516); oleylamine (August 24, 1987, 52 FR 31962 and December 1, 1988, 53 FR 48542); commercial hexane (February 5, 1988, 53 FR 3382); unsubstituted

phenylenediamines (November 30, 1989, 54 FR 49285); isopropanol (October 23, 1989, 54 FR 43252).

(b) Proposed test rule for triethylene glycol monomethyl, monoethyl, and monobutyl ether (May 15, 1986, 51 FR 17883).

(c) The TSCA health effects testing guideline for the mouse visible specific locus test (July 1, 1987, 40 CFR 798.5200)

(d) Notice containing EPA's Good Laboratory Practice Standards (August 17, 1989, 54 FR 34034).

(2) Communications, none.

This record is available for inspection in the TSCA Public Docket Office, Rm. G-004, NE Mall, 401 M St., SW., Washington, DC, from 8 a.m. to 4 p.m., Monday through Friday, except legal holidays. EPA will supplement this record with information as received.

IV. Other Regulatory Requirements

A. Executive Order 12291

Under Executive Order 12291, EPA must judge whether a rule is "major" and therefore subject to the requirement of a Regulatory Impact Analysis. EPA has determined that the amendments to these test rules would not be major because they do not meet any of the criteria set forth in section 1(b) of the Order, i.e., they would not have any annual effect on the economy of at least $100 million, would not cause a major increase in prices, and would not have a significant adverse effect on competition or the ability of U.S. enterprises to compete with foreign enterprises.

This rule was submitted to the Office of Management and Budget (OMB) for review as required by Executive Order 12291. Any written comments from OMB to EPA, and any EPA response to those comments, are included in the rulemaking record.

B. Regulatory Flexibility Act

Under the Regulatory Flexibility Act (5 U.S.C. 601 *et seq.*, Pub. L. 96-354, September 19, 1980), EPA is certifying that this rule, if promulgated, would not have a significant impact on a substantial number of small businesses because: (1) They are not likely to perform testing themselves, or to participate in the organization of the testing effort; (2) they will experience only very minor cost, if any, in securing exemption from testing requirements; and (3) they are unlikely to be affected by reimbursement requirements.

C. Paperwork Reduction Act

OMB has approved the information collection requirements contained in this rule under the provisions of the Paperwork Reduction Act of 1980, 44 U.S.C. 3501 *et seq.*, and has assigned OMB control number 2070-0033.

EPA has determined that this rule does not change existing recordkeeping or reporting requirements nor does it impose any additional recordkeeping or reporting requirements on the public.

Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M St., SW., Washington, DC 20460; and to the Office of Management and Budget, Paperwork Reduction Project (2070-0033), Washington, DC 20503.

List of Subjects in 40 CFR Parts 798 and 799

Chemical export, Chemicals, Environmental protection, Hazardous substances, Health, Laboratories, Recordkeeping and reporting requirements, Testing.

Dated: March 29, 1990.

Linda J. Fisher,

Assistant Administrator for Pesticides and Toxic Substances.

Therefore, 40 CFR, chapter I, subchapter R, is amended as follows:

PART 798 —[AMENDED]

1. In part 798:

a. The authority citation for part 798 continues to read as follows:

Authority: 15 U.S.C. 2603.

b. In subpart F by adding § 798.5195 to read as follows:

§ 798.5195 Mouse biochemical specific locus test.

(a) *Purpose.* The mouse biochemical specific locus test (MBSL) may be used to detect and quantitate mutations originating in the germ line of a mammalian species.

(b) *Definitions.* (1) A biochemical specific locus mutation is a genetic change resulting from a DNA lesion causing alterations in proteins that can be detected by electrophoretic methods.

(2) The germ line is comprised of the cells in the gonads of higher eukaryotes, which are the carriers of the genetic information for the species.

(c) *Reference substances.* Not applicable.

(d) *Test method*—(1) *Principle.* The principle of the MBSL is that heritable damage to the genome can be detected by electrophoretic analysis of proteins in the tissues of the progeny of mice treated with germ cell mutagens.

(2) *Description.* For technical reasons, males rather than females are generally treated with the test chemical. Treated males are then mated to untreated females to produce F1 progeny. Both blood and kidney samples are taken from progeny for electrophoretic analysis. Up to 33 loci can be examined by starch-gel electrophoresis and broad-range isoelectric focussing. Mutants are identified by variations from the normal electrophoretic pattern. Presumed mutants are bred to confirm the genetic nature of the change.

(3) *Animal selection*—(i) *Species and strain.* Mice shall be used as the test species. Although the biochemical specific locus test could be performed in a number of in bred strains, in the most frequently used cross, C57BL/6 females are mated to DBA/2 males to produce (C57BL/6 x DBA/2) F1 progeny for screening.

(ii) *Age.* Healthy, sexually-mature (at least 8 weeks old) animals shall be used for treatment and breeding.

(iii) *Number.* A decision on the minimum number of treated animals should take into account possible effects of the test chemical on the fertility of the treated animals. Other considerations should include:

(A) The production of concurrent spontaneous controls.

(B) The use of positive controls.

(C) The power of the test.

(4) *Control groups*—(i) *Concurrent controls.* An appropriate number of concurrent control loci shall be analyzed in each experiment. These should be partly derived from matings of untreated animals (from 5 to 20 percent ofthe treated matings), although some data on control loci can be taken from the study of the alleles transmitted from the untreated parent in the experimental cross. However, any laboratory which has had no prior experience with the test shall produce a spontaneous control sample of about 5,000 progeny animals and a positive control (using 100 mg/kg ethylnitrosourea) sample of at least 1,200 offspring.

(ii) *Historical controls.* Long-term, accumulated spontaneous control data (currently, 1 mutation in 1,200,000 control loci screened) are available for comparative purposes.

(5) *Test chemicals*—(i) *Vehicle.* When possible, test chemicals shall be dissolved or suspended in distilled water or buffered isotonic saline. Water-insoluble chemicals shall be dissolved or suspended in appropriate vehicles. The vehicle used shall neither interfere with the test chemical nor produce major toxic effects. Fresh preparations of the test chemical should be employed.

(ii) *Dose levels.* Usually, only one dose need be tested. This should be the

maximum tolerated dose (MTD), the highest dose tolerated without toxic effects. Any temporary sterility induced due to elimination of spermatogonia at this dose must be of only moderate duration, as determined by are turn of males to fertility within 80 days after treatment. For evaluation of dose-response. it is recommended that at least two dose levels be tested.

(iii) *Route of administration.* Acceptable routes of administration include, but are not limited to, gavage,inhalation, and mixture with food or water, and intraperitoneal or intravenous injections.

(e) *Test performance*—(1) *Treatment and mating.* Male DBA/2 mice shall be treated with the test chemical and mated to virgin C57BL/6 females immediately after cessation of treatment. Each treated male shall be mated to new virgin C57BL/6 females each week. Each pairing will continue for a week until the next week's mating is to begin. This mating schedule permits sampling of all post-spermatogonial stages of germ-cell development during the first 7 weeks after exposure. Spermatogonial stem cells are studied thereafter. Repeated mating cycles should be conducted until sufficient offspring have been obtained to meet the power criterion of the assay for spermatogonial stem cells.

(2) *Examination of offspring*—(i) *Birth and weaning.* Offspring shall be examined at birth and at weaning for externally detectable changes in morphology and behavior; these could be due to dominant mutations. Such characteristics may include, but are not limited to, variations in coat color, appearance of eyes, size (in which case weighing of variant animals and littermates should be carried out), fur texture, etc. Gross changes in external form and behavior shall also be sought. Scrutiny of such visible characteristics of all animals shall be made during all subsequent manipulations of the animals.

(ii) *Tissue sampling.* Blood (about 0.1 mL) and one kidney shall be removed from progeny mice under anesthesia. Both tissues are then prepared for analysis by electrophoresis.

(iii) *Electrophoresis.* The gene products of 6 loci shall be analyzed in the blood sample by broad-range isoelectric focussing and of 27 loci in the kidney sample by starch-gel electrophoresis and enzyme-specific staining. Details on these procedures are included in paragraphs (g)(1) through (g)(3) of this section.

(iv) *Mutant identification.* Presumptive electrophoretic mutants shall be identified by variation from the normal electrophoretic banding patterns. Reruns of all variant samples shall be performed to confirm the presence of altered banding patterns. Samples from parents of progeny exhibiting banding pattern variations shall be assayed to determine whether the variant was induced by the experimental treatment or was pre-existing. All treatment-induced variants are bred to determine the genetic nature of the change.

(f) *Data and reports*—(1) *Treatment of results.* Data shall be presented in tabular form and shall permit independent analysis of cell stage-specific effects, and dose-dependent phenomena. The data shall be recorded and analyzed in such a way that clusters of identical mutations are clearly identified. The individual mutants detected shall be thoroughly described. In addition, concurrent positive control data (if employed) and spontaneous control data shall also be tabulated. These concurrent controls shall be added to, as well as compared with, the historical control data.

(2) *Statistical evaluation.* Data shall be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive response, one of which is a statistically significant dose-related increase in the frequency of electrophoretic mutations. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of these test points.

(ii) A test chemical which does not produce a statistically significant increase in the frequency of electrophoretic mutations over the spontaneous frequency, or a statistically significant and reproducible positive response for at least one of the test points, is considered nonmutagenic in this system, provided that the sample size is sufficient to exclude a biologically significant increase in mutation frequency.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the MBSL indicate that, under the test conditions, the test chemical induces heritable gene mutations in a mammalian species.

(ii) Negative results indicate that, under the test conditions, the test chemical does not induce heritable genemutations in a mammalian species.

(5) *Test report.* In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, and paragraph (h) of this section, the following specific information shall be reported:

(i) Strain, age and weight of animals used; numbers of animals of each sex in experimental and control groups.

(ii) Test chemical vehicle, doses used, rationale for dose selection, and toxicity data, if available.

(iii) Route and duration of exposure.

(iv) Mating schedule.

(v) Number of loci screened for both treated and spontaneous data.

(vi) Criteria for scoring mutants.

(vii) Number of mutants found/locus.

(viii) Loci at which mutations were found.

(ix) Use of concurrent negative and positive controls.

(x) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline, the following references should be consulted:

(1) Personal communication from Susan E. Lewis, Ph.D. to Dr. Michael Cimino, U.S. EPA, OTS, October 5, 1989.

(2) Johnson, F.M., G.T. Roberts, R.K. Sharma, F.Chasalow, R. Zweidinger, A. Morgan, R.W. Hendren, and S.E.Lewis. "The detection of mutants in mice by electrophoresis: Results of a model induction experiment with procarbazine." *Genetics* 97:113–124 (1981).

(3) Johnson, F.M. and S.E. Lewis. "Mutation rate determinations based on electrophoretic analysis of laboratory mice." *Mutation Research* 82:125–135 (1981a).

(4) Johnson, F.M. and S.E. Lewis. "Electrophoretically detected germinal mutations induced by ethylnitrosourea in the mouse." *Proceedings of the National Academy of Sciences* 78:3138–93141 (1981b).

(5) Lewis, S.E., C. Felton, L.B. Barnett, W. Generoso, N. Cacheiro, and M.D. Shelby. "Dominant visible and electrophoretically expressed mutations induced in male mice exposed to ethylene oxide by inhalation." *Environmental Mutagenesis* 8:867–872 (1986).

(h) *Additional requirements.* Testing facilities conducting the mouse biochemical specific locus test in accordance with this section shall, in addition to adhering to the provisions of §§ 792.190 and 792.195 of this chapter, obtain, adequately identify, and retain for at least 10 years, acceptable 35–mm photographs (and their negatives) of the stained isoelectric-focussing columns and the stained starch-gels obtained following analyses of blood and kidney preparations, respectively, from mutant mice, their siblings, and their parents.

c. In § 798.5200 by revising paragraph (f)(5) and adding paragraph (h) to read as follows:

§ 798.5200 Mouse visible specific locus test.

* * * * *

(f) * * *

(5) *Test report.* In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, and paragraph (h) of this section, the following specific information shall be reported:

* * * * *

(h) *Additional requirements.* Testing facilities conducting the mouse visible specific locus test in accordance with this section shall, in addition to adhering to the provisions of §§ 792.190 and 792.195 of this chapter, obtain, and retain for at least 10 years, acceptable 35–mm color photographs (and their negatives) demonstrating the visible mutations observed in mutant animals and the lack of such mutations in their siblings and parents.

PART 799—[AMENDED]

2. In part 799:

a. The authority citation for part 799 continues to read as follows:

Authority: 15 U.S.C. 2603, 2611, 2625.

b. In § 799.1700 by revising paragraphs (c)(1)(i)(C)(*1*), (c)(1)(ii)(A), and (d) to read as follows:

§ 799.1700 Fluoroalkenes.

* * * * *

(c) * * *

(1) * * *

(i) * * *

(C) * * *

(*1*) A mouse visible specific locus assay (MVSL) shall be conducted with VF, VDF, TFE, and HFP in accordance with § 798.5200 of this chapter, except for the provisions of paragraph (d)(5) of § 798.5200, or a mouse biochemical-specific locus assay (MBSL) shall be conducted with VF, VDF, TFE, and HFP in accordance with § 798.5195 of this chapter, except for the provisions of paragraph (d)(5) of § 798.5195, for whichever of these substances produces a positive test result in the sex-linked recessive lethal test in *Drosophila melanogaster* conducted pursuant to paragraph (c)(1)(i)(B) of this section if, after a public program review, EPA issues a **Federal Register** notice or sends a certified letter to the test sponsor specifying that the testing shall be initiated.

* * * * *

(ii) * * *

(A) Mutagenic effects-gene mutation tests shall be completed and the final reports shall be submitted to EPA as follows: Somatic cells in culture assay, within 6 months after the effective date of the final rule; *Drosophila* sex-linked recessive lethal, within 9 months (for VF and VDF) and within 15 months (for TFE and HFP) after the effective date of the final rule; MVSL or MBSL, within 51 months after the date of EPA's notification of the test sponsor by certified letter or **Federal Register** notice that testing shall be initiated.

* * * * *

(d) *Effective dates.* (1) The effective date of this rule is July 22, 1987, except for the provisions of paragraphs (c)(1)(i)(C)(*1*), and (c)(1)(ii)(A), which are effective May 21, 1990.

(2) The guidelines and other test methods cited in this section are referenced as they exist on the effective date of the final rule.

* * * * *

c. In § 799.2155 by adding paragraphs (c)(5)(i)(D); (c)(5)(ii)(A)(*4*), and (c)(5)(ii)(C); and revising paragraph (d) to read as follows:

§ 799.2155 Commercial hexane.

* * * * *

(c) * * *

(5) * * *

(i) * * *

(D)(*1*) Unless the results of the sex-linked recessive lethal test in *Drosophila melanogaster* conducted with commercial hexane pursuant to paragraph (c)(5)(i)(C) of this section are negative, EPA shall conduct a public program review of all of the mutagenicity data available for this substance. If, after this review, EPA decides that testing of commercial hexane for causing heritable gene mutations in mammals is necessary, it shall notify the test sponsor by certified letter or **Federal Register** notice that testing shall be initiated in either the mouse visible specific locus test or the mouse biochemical specific locus test. The mouse visible specific locus test, if conducted, shall be performed for commercial hexane in accordance with § 798.5200 of this chapter except for the provisions in paragraphs (d)(5)(ii) and (d)(5)(iii) of § 798.5200. The mouse biochemical specific locus test, if conducted, shall be performed for commercial hexane in accordance with § 798.5195 of this chapter except for the provisions in paragraphs (d)(5)(ii) and (d)(5)(iii) of § 798.5195.

(*2*) For the purposes of this section, the following provisions also apply:

(*i*) *Dose levels.* A minimum of two dose levels shall be tested. The highest dose tested shall be the highest dose tolerated without toxic effects, provided that any temporary sterility induced due to elimination of spermatogonia is of only moderate duration, as determined by a return of males to fertility within 80 days of treatment, or shall be the highest dose attainable below the lower explosive limit concentration of commercial hexane. Exposure shall be for 6 hours a day. Duration of exposure shall be dependent upon the accumulated total dose desired for each group.

(*ii*) *Route of administration.* Animals shall be exposed to commercial hexane by inhalation.

(ii) * * *

(A) * * *

(*4*) The mouse visible specific locus test or the mouse biochemical specific locus test shall be completed and a final report shall be submitted to EPA within 51 months of the date on which the test sponsor is notified by EPA by certified letter or **Federal Register** notice that testing shall be initiated.

* * * * *

(C) Interim progress reports for either the mouse visible specific locus test or the mouse biochemical specific locus test shall be submitted to EPA at 6–month intervals, beginning 6 months after EPA's notification of the test sponsor that testing should be initiated, until the applicable final report is submitted to EPA.

* * * * *

(d) *Effective dates.* (1) The effective date of this final rule is November 17, 1988, except for the provisions of paragraphs (c)(5)(i)(D), (c)(5)(ii)(A)(*4*), and (c)(5)(ii)(C), which are effective May 21, 1990.

(2) The guidelines and other test methods cited in this section are referenced as they exist on the effective date of the final rule.

* * * * *

d. In § 799.3175 by revising paragraphs (c)(3)(i)(C) and (d), and adding paragraphs (c)(3)(ii)(C), (c)(3)(iii)(A)(*3*), and (c)(3)(iii)(C) to read as follows:

§ 799.3175 Oleylamine.

* * * * *

(c) * * *

(3) * * *

(i) * * *

(C) A mouse visible specific locus test (MVSL) or a mouse biochemical specific locus test (MBSL) shall be conducted for ODA if it produces a positive result in the sex-linked recessive lethal test in *Drosophila melanogaster* conducted pursuant to paragraph (c)(3)(i)(B) of this section and if so required in a **Federal**

Register notice or certified letter sent to test sponsors.

* * * * *

(ii) * * *

(C)(*1*) If required, the MVSL or MBSL shall be conducted with ODA in accordance with §§ 798.5200 or 798.5195 of this chapter, respectively, except for the provisions of paragraph (d)(5)(iii) of each of these sections.

(*2*) For purposes of this section, the following provision also applies.

(*i*) *Route of administration.* The route of exposure shall be oral by gavage.

(*ii*) [Reserved]

(iii) * * *

(A) * * *

(*3*) The MVSL or MBSL shall be completed and the final report submitted to EPA within 51 months of EPA'snotification of the test sponsor by certified letter or **Federal Register** notice that testing shall be initiated.

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(C) Progress reports shall be submitted to EPA for the MVSL or the MBSL at 6-month intervals, the first of which is due within 6 months of EPA's notification of the test sponsor that testing shall be initiated.

* * * * *

(d) *Effective dates.* (1) The effective date of this rule is October 7, 1987, except for the provisions of paragraphs (c)(1)(ii) and (c)(1)(iii), (c)(2)(ii) and (c)(2)(iii); (c)(3)(ii)(A), and (c)(3)(ii)(B), (c)(3)(iii)(A)(*1*), (c)(3)(iii)(A)(*2*), (c)(3)(iii)(B), (c)(4)(ii) and (c)(4)(iii), which are effective on January 17, 1989.

(2) Paragraphs (c)(3)(i)(C), (c)(3)(ii)(C), (c)(3)(iii)(A)(*3*), and (c)(3)(iii)(C) of this rule are effective May 21, 1990.

(3) The guidelines and other test methods cited in this section are referenced as they exist on the effective date of the final rule.

* * * * *

e. In § 799.2325 by revising paragraphs (c)(5)(i)(C)(*1*), (c)(5)(ii)(A)(*3*), and (d) to read as follows:

§ 799.2325 Isopropanol.

* * * * *

(c) * * *

(5) * * *

(i) * * *

(C) * * *

(*1*) The mouse visible specific locus test (MVSL) shall be conducted with isopropanol by inhalation in accordance with § 798.5200 of this chapter, except for the provisions in paragraphs (d)(5)(ii) and (d)(5)(iii) of § 798.5200, or a mouse biochemical specific locus test (MBSL) shall be conducted with isopropanol by inhalation in accordance with § 798.5195 of this chapter, except for the provisions in paragraphs (d)(5)(ii) and (d)(5)(iii) of § 798.5195, if the results of the sex-linked recessive lethal test conducted pursuant to paragraph (c)(4)(i)(B) of this section are positive and if, after a public program review, EPA issues a **Federal Register** notice or sends a certified letter to the test sponsor specifying that the testing shall be initiated.

* * * * *

(ii) * * *

(A) * * *

(*3*) The MVSL or MBSL test within 51 months of the date of EPA's notification of the test sponsor by certified letter or **Federal Register** notice under paragraph (c)(4)(i)(C) of this section that testing shall be initiated.

* * * * *

(d) *Effective dates.* (1) The effective date of this rule is December 4, 1989, except for the provisions of paragraphs (c)(5)(i)(C)(*1*), and (c)(5)(ii)(A)(*3*), which are effective May 21, 1990.

(2) The guidelines and other test methods cited in this section are referenced as they exist on the effective date of the final rule.

* * * * *

f. In § 799.3300 by revising paragraphs (c)(1)(i)(B), (c)(1)(ii)(C), (c)(1)(ii)(F), and (f) to read as follows:

§ 799.3300 Unsubstituted phenylenediamines.

* * * * *

(c) * * *

(1) * * *

(i) * * *

(B) If the SLRL assay conducted pursuant to paragraph (c)(1)(i)(A) of this section is positive, either the mouse visible specific locus test (MVSL) or the mouse biochemical specific locus test (MBSL) shall be conducted for *m*-pda by gavage in accordance with §§ 798.5200 or 798.5195 of this chapter, if after public program review, EPA issues a **Federal Register** notice or sends a certified letter to the test sponsor(s) specifying that testing shall be initiated. The test sponsor shall notify EPA of its choice in writing in its first interim report.

* * * * *

(ii) * * *

(C) If required, the MVSL or the MBSL shall be completed and the final report shall be received by EPA no later than 51 months after EPA issues a **Federal Register** Notice or sends a certified letter to the test sponsor(s) identified under paragraph (c)(1)(i)(B) of this section specifying that testing shall be initiated.

* * * * *

(F) Interim reports for the HT and either the MBSL or MVSL are required at 6-month intervals beginning 6 months after the date of notification by EPA that testing shall be initiated, and ending when the final report is submitted.

* * * * *

(f) *Effective date.* (1) The effective date of this rule is January 16, 1990, except for the provisions of paragraphs (c)(1)(i)(B), (c)(1)(ii)(C), and (c)(1)(ii)(F), which are effective on May 21, 1990.

(2) The guidelines and other test methods cited in this section are referenced as they exist on the effective date of the final rule.

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